

CLAIMS

1. Nucleotide sequences, characterised in that they are isolated from their natural environment and in that they contain at least a part of the sequence SEQ ID ~~No:~~ 1, one or more nucleotides being modified if necessary, it being understood that said sequences are capable of coding for polypeptides having lectinic properties.

2. Nucleotide sequences, characterised in that they are isolated from their natural environment and in that they are capable of coding, when used according to conventional recombinant DNA techniques, for sarcolectins containing at least one amino acid chain as indicated in SEQ ID ~~No:~~ 1, in which one or more amino acids are modified, if necessary.

3. Nucleotide sequences, characterised in that they are isolated from their natural environment and in that they are capable of hybridising with at least one fragment of SEQ ID ~~No:~~ 1 carrying at least part of the genetic information for a sarcolectin.

4. Nucleotide sequences according to ~~one of claims 1 to 3~~, characterised in that it corresponds to the open reading frame of 1407 bp running from position 62 in SEQ ID ~~No:~~ 1 to position 1469.

5. Nucleotide sequences according to ~~one of claims 1 to 4~~, characterised in that they contain at least part of region 5' of SEQ ID ~~No:~~ 1 and/or of region 3'.

6. Nucleotide sequences according to claim 5, characterised in that they contain at least part of the sequence SEQ ID ~~No:~~ 2, one or more nucleotides being modified, if necessary.

7. mRNA sequences corresponding to the sequences according to ~~one of claims 1 to 6~~.

8. The antisense sequences corresponding to the sequences according to ~~one of the preceding claims~~.

9. The cDNA corresponding to the sequences according to ~~one of claims 1 to 8~~.

10. Expression vectors containing at least one of the nucleotide sequences according to ~~one of the preceding claims~~, under the control of a suitable promoter.

11. Host cells transfected by the vectors according to claim 10, containing the recombinant proteins expressed.

12. Novel compounds, characterised in that they have a lectinic activity and contain at least a part of an amino acid chain in the sequence coded by ~~one of the nucleotide sequences defined in one of claims 1 to 6~~, one or more amino acids being modified, if necessary, so long as said modification does not adversely affect the lectinic properties of the sarcolectins.

13. Novel compounds, characterised in that they have an amino acid chain in SEQ ID ~~N°~~ 3 or SEQ ID N° 4.

14. Novel compounds, characterised in that they correspond to the sequence coded by the open reading frame of SEQ ID ~~N°~~ 1 and contain 469 amino acids, their molecular weight being evaluated at approximately 55 kd.

15. Novel compounds, characterised in that they are compounds of the kind obtained by expression, in a suitable host cell according to recombinant DNA techniques, of an expression vector containing a DNA sequence as defined in ~~one of claims 1 to 6~~, recovery of the compound expressed and purification.

16. Novel compounds, characterised in that they are fragments or derivatives of the compounds according to ~~one of claims 12 to 15~~, so long as they retain lectinic properties, at least in part.

17. Novel compounds according to claim 16, characterised in that they are the SEQ ID ~~N°~~ 5 peptides.

18. Novel compounds according to claim 17, characterised in that they are peptides having the sequence of fragment 41 to 55 in SEQ ID ~~N°~~ 1, or peptide derivatives of this sequence and characterised in that they are recognised by monoclonal antibodies capable of reacting with

SEQ ID <sup>NB</sup> 1, by their own antibodies, but that they do not react with antibodies directed against the peptide fragment 81 to 95 in SEQ ID <sup>NB</sup> 1.

19. Novel compounds according to claim 16, characterised in that they are the SEQ ID <sup>NB</sup> 6 peptides.

20. Novel compounds according to claim 17, characterised in that they are peptides having the sequence of fragment 81 to 95 in SEQ ID <sup>NB</sup> 1, or peptide derivatives of this sequence and characterised in that they are recognised by monoclonal antibodies capable of reacting with SEQ ID <sup>NB</sup> 1, by their own antibodies, but in that they do not react with antibodies directed against the peptide fragment 41 to 55 in SEQ ID <sup>NB</sup> 1.

21. Compounds according to claim 12, as obtained by purification on the basis of tissue extracts, by a process comprising:

- treatment of the tissue extract containing lectins under controlled conditions with pepsin or at an acid pH, under conditions which make it possible to remove at least the majority of the contaminating proteins whilst retaining the lectinic activity,

- chromatography on Sephadryl S-200 and, if necessary, DEAE cellulose

- chromatography on CM-Trisacryl-M

- affinity chromatography using a sugar such as N-acetylneuraminic acid as ligand, and

- separation by reversed-phase HPLC.

22. Compounds according to claim 21, characterised in that the fraction separated by reversed-phase HPLC undergoes electrophoresis on SDS-PAGE gel and denaturation and in that the bands corresponding to 55 kd and ≤ 14 kd are recovered.

23. The antibodies directed against the compounds according to <sup>Claim 12</sup> one of claims 12 to 22.

24. Antibodies according to claim 23, characterised

Mb A17

*MC*

in that they are antibodies directed against the 55 kd protein according to claim 22 and in that they recognise the 65 kd protein as well as the 55 kd protein.

25. A process for obtaining compounds according to claim 12 or 21 with a high degree of purity comprising the treatment of a tissue extract containing sarcolectin with pepsin or at an acid pH so as to remove at least the majority of the contaminating proteins from the extract whilst retaining the lectinic biological activity, and the removal of impurities by chromatography on Sephadryl S-200 and DEAE cellulose, characterised in that it also comprises a stage of chromatography on CM-Trisacryl-M, then affinity chromatography using N-acetylneuraminic acid as ligand, the purity of the SCLs being verified by HPLC if desired.

26. A process according to claim 25, characterised in that the stage of chromatography on CM-Trisacryl-M is carried out with the aid of a first buffer in order to remove at least the majority of the contaminating albumin, then a second buffer in order to elute the SCLs retained on the column.

27. A process according to claim 25 or 26, characterised in that the stage of affinity chromatography carried out with a sugar as ligand comprises the use of an agarose gel column on which is fixed the sugar and at least two buffers, so as to elute firstly the SCLs then to remove the contaminating proteins.

28. A process according to *one of claims 25 to 27*, characterised in that the separation by HPLC is carried out with the aid of a water/acetonitrile/ trifluoroacetic acid system.

29. A process according to claim 28, characterised in that the fraction corresponding to the main peak in the HPLC stage undergoes electrophoresis on SDS-PAGE gel, and denaturation and in that the 65 kd, 55 kd and ≤ 14 kd bands respectively are recovered.

*Claim 25*

30. A process for obtaining sarcolectins with a high degree of purity, characterised in that it comprises:

- lowering the pH of the medium to pH 5 for 30 minutes;
- lowering the pH to 5 which leads to a substantial precipitate which will be removed by centrifugation;
- readjustment to pH 7.4 and recovery of the supernatant which contains the 65 and 55 kd proteins which can be recognised by Western blots with the aid of specific antibodies.

31. Growth factors, which can be used more particularly in order to contribute to the regeneration of damaged tissues and to an improvement in the wound healing process, characterised in that they are SCL according to Claim 15 or claims 12 to 22.

32. Therapeutic agents for stimulating the immune system, particularly of specific immunity, characterised in that they are SCL according to Claim 12 or claims 12 to 22, if necessary in association with specific growth factors such as interleukin-2.

33. The use of the SCLs according to one of claims 12 to 22 in order to select inhibitors of their lectinic activity such as sugars, aminobutyric acids, and possibly interferons in a high dose.

34. Agents capable of blocking the action of the SCLs produced in excess in pathological states, such as cancers, chronic viral or autoimmune diseases, characterised in that they are antibodies according to one of claims 23 or 24.

35. An in vitro method for the bioassay for SCL, characterised in that it comprises

- bringing a biological sample to be analysed or cells into contact with an anti-SCL antibody preparation according to claim 21, or an antibody fragment, the antibody used being immobilised on a solid support, under suitable

conditions for the production of an antigen-antibody complex with the SCLs if they are present in the sample or the cells.

- detecting the formation of such a complex of the antigen-antibody type.

36. A kit for the diagnosis of the presence of SCL in a biological sample or cells, characterised in that it comprises

- a suitable solid phase acting as a support

- a preparation of anti-SCL antibodies according to claim 24 or of free or immobilised fragments directed against the peptides 41-55

- buffer solutions and suitable reagents for immunological reactions and for detection reactions.

37. A method for in vitro detection of SCLs, characterised in that it comprises

- bringing a biological sample to be analysed or cells into contact with a probe developed from a fragment of nucleotides according to <sup>Claim 1</sup> ~~one of claims 1 to 7 or 9~~, under suitable conditions for the production of a hybridisation complex with the DNAs coding for SCLs if they are present in the sample or cells, and

- detecting the formation of the hybridization complex if the SCLs are present in the sample or the cells.

38. A kit for the in vitro detection of the presence of genes coding for SCLs, characterised in that it comprises:

- nucleotide probes developed from the sequences defined in <sup>Claim 1</sup> ~~one of claims 1 to 7 or 9~~, and

- buffer solutions and reagents for the hybridisation reaction.

39. The use of the antisense sequences according to claim 8 for blocking the expression of SCL according to one of claims 12 to 22.

40. The use of a compound according to one of claims

- 70 -  
69

12 to 20 fixed to albumin in order to produce a retard [sic] vehicle.

41. The use of a compound according to any one of claims 12 to 22, in particular of human recombinant products, as a growth factor for cells in vitro.

PL  
C30

add  
F3

8600520 3094260